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Boehringer Mannheim I

Proteases for Cleavage and Sequencing

Proteases continued

Product	Application	Specificity
Thrombin Coagulation factor IIa from human plasma lyophilizate *EC 3.4.21.5* anti-HIV	Coagulation research, medical research, protein structure analysis and biochemical research	Serine endopeptidase hydrolyzing peptide and ester bonds specifically at the carboxylic side of Arg.
Trypsin from bovine pancreas *EC 3.4.21.4*	Protein degradation and tissue dissociation	Serine endopeptidase hydrolyzing specifically proteins and peptides at the carboxylic side of the basic amino acids Arg and Lys. Amide and ester bonds of Arg and Lys are cleaved as well.

A free Poster "Products for Protein Biochemistry" is available on request from your local representative.

anti-HIV Tested for HBs antigen and for the presence of antibodies to HIV-1, HIV-2, HCV and found to be negative. See also page XIV.

Proteases Sequencing grade

- *Proteases, see pages 400 – 406*
- *Proteases and restriction proteases for the cleavage of fusion proteins, see pages 397 – 399*
- *Protease inhibitors, see pages 484 – 490*
- *Protease substrates, see pages 499 – 500*
- *Proteases for coagulation research, see page 588*

Product	Application	Specificity
Acylamino Acid Peptidase Sequencing Grade from horse liver lyophilizate Acylamino-acid releasing enzyme EC 3.4.19.1	Deblocking of peptides for subsequent N-terminal sequence analysis	Exopeptidase releasing N-acyl amino acids from peptides and proteins: acyl-X-↓-Y- (X=preferentially Ser, Ala or Met; Y=unspecific amino acid). The specificity is tested with α-MSH as substrate
Carboxypeptidase P Sequencing Grade from *Penicillium janthinellum* lyophilizate Peptidyl-L-amino-acid hydrolase EC 3.4.16.1	Protein structure and sequence analysis	Serine carboxypeptidase hydrolyzing amino acid residues (including Pro, Asp, Glu) from the C-termini of proteins and peptides. Release of Ser and Gly is considerably retarded
Carboxypeptidase Y Sequencing Grade from yeast lyophilizate Peptidyl-L-amino-acid hydrolase EC 3.4.16.1	Sequence analysis and limited hydrolysis of peptides and proteins, especially in combination with carboxypeptidases A and B	Serine carboxypeptidase hydrolyzing amino acids (including Pro) from the C-termini of proteins and peptides. High catalysis rate if the penultimate and/or terminal amino acid carries aromatic or aliphatic side chains. The release of Gly and Asp is considerably retarded. Dipeptides are completely resistant to cleavage
Cathepsin C Sequencing Grade from bovine spleen solution Dipeptidyl peptidase Dipeptidyl transferase EC 3.4.14.1	Processing of fusion proteins. Catalysis of dipeptide transfer (transamidation)	Cysteine protease catalyzing the successive removal of N-terminal dipeptides from polypeptides. The reaction rate is dependent on the penultimate amino acid, whereby hydrophilic and hydrophobic residues are accepted. Degradation is blocked by N-terminal Lys or Arg. Pro as the second or third amino acid prevents also cleavage

For most products bulk quantities are available. Please inquire

To place an order: 0130-2228
0621/759-8545
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For technical inquiries: 0621/759-8568

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Proteases for Cleavage and S...

Characteristics	Inhibitors	Cat. No.	Pack Size	
Specific activity: approx. 120 U/mg enzyme protein at 25°C with Chromozym® TH as substrate. **Contaminants:** < 3% factor Xa **Molecular weight:** approx. 33.6 kD **pH-Optimum:** 8.2–9.0	DFP, TLCK, PMSF, benzamidine, α_1-antitrypsin, α_2-macroglobulin, antithrombin III-heparin, hirudin and APMSF	602 400	20 U	
Specific activity: approx. 110 U/mg lyophilizate at 25°C with Chromozym TRY® as substrate (approx. 40 U/mg lyophilizate at 25°C with BAEE as substrate. **Formulation:** salt-free lyophilizate **Molecular weight:** 23.5 kD **pH-Optimum:** 8.0	TLCK, DFP, PMSF, leupeptin, soybean trypsin inhibitor, trypsin inhibitor from hen egg, aprotinin, α_2-macroglobulin, α_1-antitrypsin, APMSF and antipain	109 819 109 827	500 mg 2 g	

Proteases Sequencing grade

3

Characteristics	Inhibitors	Cat. No.	Pack Size	Price
Purity: free of impurities which might interfere with the specific cleavage and/or with the separation of peptides in reversed phase HPLC **Molecular weight:** 80 kD **pH-Optimum:** 7.5–9.0	DFP	1 970 502	2 x 30 µg	596,-
Purity: free of impurities, which might interfere with amino acid analysis. 10 µg carboxypeptidase P sequencing grade contain < 10 pmol of each amino acid. Function and purity are checked by amino acid analysis and SDS-PAGE. **Molecular weight:** 91 kD **pH-Optimum:** 3.7–5.2	DFP, iodoacetic acid and p-mercuribenzoate	1 420 321 1 111 906	20 µg 3 x 20 µg	181,- 415,-
Purity: free of impurities, which might interfere with the amino acid analysis. 10 µg carboxypeptidase Y sequencing grade contain < 10 pmol of each amino acid. Function and purity are checked by amino acid analysis and SDS-PAGE. **Molecular weight:** 61 kD **pH-Optimum:** approx. 5.5 for acidic and 7.0 for basic amino acids	DFP, PMSF, ZPCK, 4-hydroxymercuri-benzoate and aprotinin	1 420 348 1 111 914	20 µg 3 x 20 µg	161,- 415,-
Purity: highly pure, not standardized with albumin **Molecular weight:** 210 kD **pH-Optimum:** 4.0–6.0 for hydrolytic activity; 7.0–8.0 for transferase/ transamidase activity	Iodoacetate and formaldehyde	1 559 621 1 559 630	3 x 30 µg (3 x 30 µg) 3 x 250 µg (3 x 250 µg)	323,- 1 188,-

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Proteases for Cleavage and Sequencing

Proteases

➲ *Proteases sequencing grade, see pages 408 – 410*
➲ *Proteases for the cleavage of fusion proteins, see pages 397 – 398*
➲ *Protease inhibitors, see pages 484 – 490*
➲ *Protease substrates, see pages 499 – 500*
➲ *Proteases for coagulation research, see page 588*
➲ *Collagenases, dispases and trypsins for tissue dissociation, see pages 200 – 204*

Product	Application	Specificity
Aminopeptidase M from pig kidney suspension in ammonium sulfate solution α-Aminoacyl-peptide hydrolase (microsomal) *EC 3.4.11.2*	Study of protein sequences and identification of chemically modified amino acid residues in proteins	Metalloprotease, hydrolyzing completely peptides and proteins with a free α-amino group and L-amino acids. X-Pro bonds are not cleaved. The amino group of Asp, Gln or β-Ala is not cleaved even after prolonged incubation
Carboxypeptidase A from bovine pancreas suspension in water Peptidyl-L-amino-acid hydrolase *EC 3.4.17.1*	Sequence analysis of proteins by successive cleavage of amino acids from the C-terminus of proteins	Zn-metalloprotease, catalyzing the release of C-terminal amino acid residues which possess a L-configuration and an unsubstituted α-amino group. Very slow release of Gly, Asp, Glu, Cys and $CysSO_3H$, no release of Arg, Pro and hydroxyproline
Carboxypeptidase B from pig pancreas solution Peptidyl-L-lysine (-L-arginine) hydrolase *EC 3.4.17.2*	Sequence analysis of proteins by successive cleavage of basic amino acids from the C-terminus of proteins	Zn-metalloprotease catalyzing the hydrolysis of the basic amino acids L-Lys and L-Arg from the C-terminal position in polypeptides.
Carboxypeptidase Y from yeast lyophilizate Peptidyl-L-amino acid hydrolase *EC 3.4.16.1* ➲ *Carboxypeptidase Y Sequencing Grade see page 406*	Sequence analysis and limited hydrolysis of peptides and proteins, especially in combination with carboxypeptidases A and B	Serine carboxypeptidase hydrolyzing L-amino acids (including Pro) from the C-termini of proteins and peptides. High catalysis rate, if the penultimate and/or terminal residue is an aromatic or aliphatic amino acid. The release of Gly and Asp is considerably retarded. Terminal Pro and β-Ala are good substrates. Dipeptides are completely resistant to cleavage
Chymotrypsin α-Chymotrypsin from bovine pancreas salt-free lyophilizate *EC 3.4.21.1* ➲ *Chymotrypsin Sequencing Grade see page 408*	Hydrolysis of proteins by chymotrypsin alone or in combination with other proteases. Suitable for peptide mapping, fingerprinting and sequence analysis	Serine endopeptidase, specifically hydrolyzing peptide bonds at the C-terminus of Tyr, Phe and Trp. Leu, Met, Ala, Asp and Glu are cleaved at lower rates. Acts also upon amides and esters of susceptible amino acids and is used for peptide synthesis
Elastase from pig pancreas lyophilizate *EC 3.4.21.36*	Digestion of elastin; tissue dissociation together with collagenase and trypsin and solubilization of membrane proteins. Hydrolyzes furthermore fibrin, casein, denatured collagen (but no native collagen), albumin, proteins from soybean and various synthetic substrates. Cleaves preferentially adjacent to neutral amino acids	Serine endopeptidase, hydrolyzing peptide bonds at the C-terminal site of amino acids with uncharged non-aromatic side chains like Ala, Val, Leu, Ile, Gly and Ser.
Endoproteinase Arg-C from mouse submaxillarie glands lyophilizate ➲ *Endoproteinase Arg-C Sequencing Grade see page 408*	Protein structure and sequence analysis	Serine protease, hydrolyzing specifically peptide and ester bonds at the carboxylic side of Arg.

Characteristics	Inhibitors	Cat.No.	Pack Size		Price
Specific activity: approx. 4 U/mg at 25°C (11 U/mg at 37°C) with leucine-4-nitranilide as substrate **Molecular weight:** 280 kD (two identical subunits) **pH-Optimum:** 7.0–7.5; up to 9.0, depending on the substrate concentration	Leucinethiol, 2,2'-bipyridine and 1,10-phenanthroline	102 768	20 U (1 ml)	DM	148,-
Specific activity: approx. 35 U/mg at 25°C with hippuryl-L-phenylalanine as substrate **Contaminants:** < 0.1% chymotrypsin, < 0.1% trypsin **Molecular weight:** 34.5 kD **pH-Optimum:** approx. 7.5, may vary with different substrates	Chelating agents like pyrophosphate, oxalate, citrate, cysteine and 1,10-phenanthroline. Freezing and lyophilization inactivates the enzyme	103 225	25 mg (1 ml)		169,-
Specific activity: approx. 150 U/mg at 25°C with hippuryl-L-arginine as substrate **Contaminants:** < 5 mU chymotrypsin/mg protein, < 0.7 mU trypsin/mg protein, < 2% carboxypeptidase A. Potential chymotrypsin and trypsin activities are eliminated by DFP treatment. **Stability:** a decrease in activity of approx. 10% may occur within 6 months **Molecular weight:** 34.6 kD **pH-Optimum:** 7.0–9.0	Chelating agents and basic amino acids	103 233	5 mg (1 ml)		193,-
Specific activity: approx. 20 U/mg lyophilizate at 37°C with Z-Phe-Ala as substrate **Stability:** a decrease in activity of approx. 10% may occur within 6 months **Molecular weight:** 61 kD **pH-Optimum:** approx. 5.5 for acidic and 7.0 for basic amino acids	DFP, PMSF, ZPCK, 4-hydroxymercuri-benzoate and aprotinin	238 139	10 mg lyophilizate		380,-
Specific activity: approx. 90 U/mg lyophilizate at 25°C with acetyl-L-tyrosine ethyl ester as substrate **Preparation:** from activated, crystallized chymotrypsinogen A **Molecular weight:** 25 kD **pH-Optimum:** 7.0–9.0	Aprotinin, DFP, PMSF, phenothiazine-N-carbonyl chloride, TPCK, ZPCK, α-macroglobulin, α_1-antitrypsin, soybean trypsin inhibitor and chymostatin; not inhibited by APMSF	103 306 103 314	500 mg 1 g		58,- 82,-
Specific activity: approx. 130 U/mg protein (approx. 105 U/mg lyophilizate) at 25°C with N-acetyl-trialanyl-methyl ester as substrate **Molecular weight:** 25.9 kD **pH-Optimum:** 8.8	DFP, α_1-antitrypsin, α_2-macroglobulin, 4-dinitrophenol diethyl-phosphate, PMSF, 3,4-dichloroisocoumarin, elastatinal	1 027 891 1 027 905	10 mg 50 mg		148,- 465,-
Specific activity: approx. 220 U/mg protein (approx. 20 U/mg lyophilizate) at 25°C with N-tosyl-L-arginine-methylester as substrate **Molecular weight:** 90 kD **pH-Optimum:** 8.0–8.5	DFP, α_2-macroglobulin and TLCK	289 590	100 U		211,-

[redacted] SGE-ST. FORSCH.

Art. 8444 dt. engl.

Trypsin

(aus Schweinepankreas) lyophilisiert

gereinigt durch Affinitätschromatographie
für die Proteinsequenzanalyse

[redacted]g enthält 5 × 50 µg

Spezifikation

50 µg Lyophilisat enthalten ca. 2,5 Einheiten Trypsin (Insulin B_{ox}, 25 °C, 1 h, pH 8,5)

Abwesenheit von Chymotrypsin und anderen Fremdprotease-Aktivitäten nachgewiesen durch RP-HPLC mit Insulin B_{ox}

Einheitendefinition

1 Einheit ist definiert als diejenige Enzym-Menge, die notwendig ist, um 1 mg Insulin B_{ox} in 1 Stunde bei 25 °C und pH 8,5 vollständig zu spalten.

Spezifität

Trypsin für die Proteinsequenzanalyse spaltet bei pH 7,5 bis 9,0 spezifisch Peptidbindungen C-terminal an Lysin und Arginin.

Stabilität

Stabil bei —20 °C, trocken gelagert. Eine Lösung (z.B. in steriliem. bidest. Wasser) ist bei —20 °C gelagert mindestens 4 Wochen ohne Einfluß auf die Spezifität stabil.

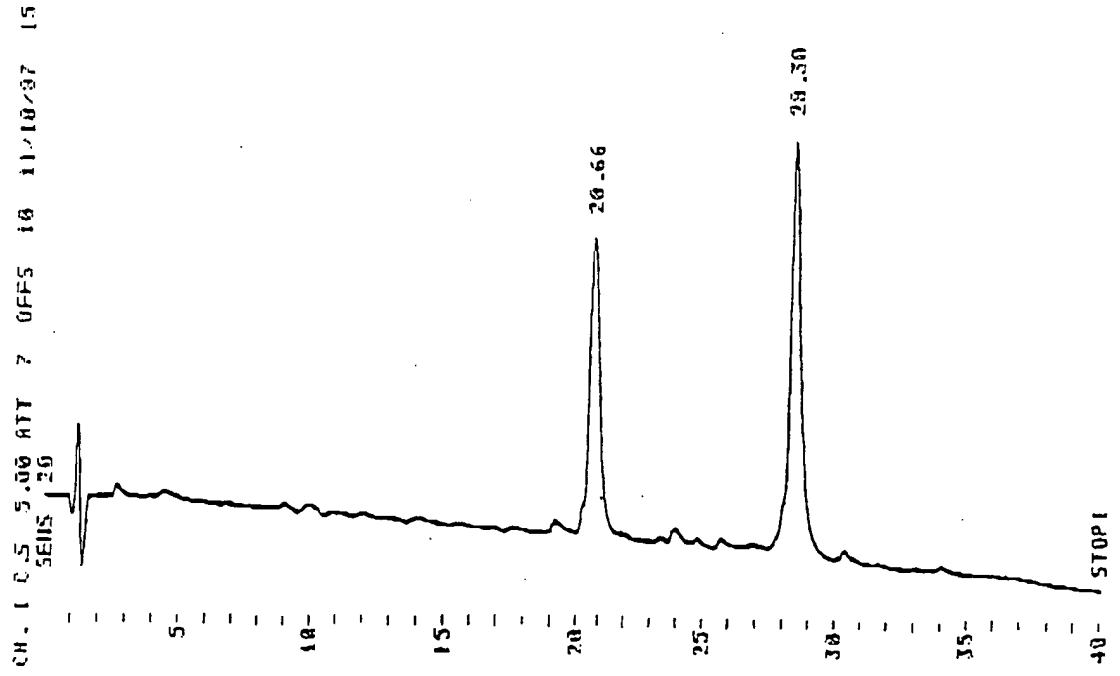
Spezifitätstest

Die Überprüfung der Spezifität erfolgt durch Verdauung von Insulin B_{ox} und anschließender „reversed phase" HPLC.

Verdauungsansatz: 0,09 mg Insulin B_{ox}
0,05 mg Trypsin
100 µl Tris/HCl-Puffer 50 mmol/l, pH 8,5

Inkubation 1 Stunde bei 25 °C

Chromatographie:
Säule: LiChrospher 60 Select B (5 µm)
Auftrag: 85 µl Verdauungsansatz
Gradienten-Lösung A: 10 % Acetonitril 90 % TFA (0,1 % v/v in Wasser)
Gradienten-Lösung B: 90 % Acetonitril 10 % TFA (0,1 % v/v in Wasser)
Gradient: 35 min linear 0—35 % B
Durchflußgeschwindigkeit: 1 ml/min
Detektion: 215 nm



E. Merck, Postfach 4119, D-6100 Darmstadt 1, F.R. Germany
Tel.: (06151) 720, Telex 419328-0 em d

Trypsin

sequencing grade

aus Rinderpankreas

4 x 100 µg – Best. Nr. 1047841

Handelsform: Lyophilisat, salzfrei.

Trypsin sequencing grade wird als spezifische Protease in hochreiner Form aus Rinderpankreas isoliert.

Reinheit: Das Enzym ist frei von Verunreinigungen, die im Trennbereich von Peptiden bei "reversed phase" HPLC (höchstempfindliche Detektion bei 206–230 nm) interferieren können. Funktions- und Reinheitskontrolle mittels HPLC bei jeder Charge garantieren gleichbleibende Qualität (Abb. 1).

Spezifität: *Trypsin sequencing grade* ist eine Serin-Protease. Sie spaltet bei pH = 7,5–9 spezifisch Peptidbindung C-terminal an Lysin und Arginin.
Die Spezifität von *Trypsin sequencing grade* wird mit Insulin, Kette B oxidiert (Insulin B_{ox}) als Substrat überprüft. Dabei wird *Trypsin sequencing grade* in hoher Konzentration (1 Gewichtsteil *Trypsin sequencing grade* mit 18 Gewichtsteilen Insulin B_{ox}) 18 Stunden inkubiert, um auch geringe Verunreinigungen durch Chymotrypsin zu erkennen (Abb. 2).

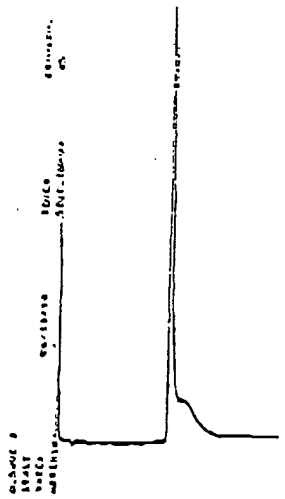


Abb. 1: Reinheit von *Trypsin sequencing grade* in der „reversed phase" HPLC.
Menge: 20 µg *Trypsin sequencing grade*; Volumen: 20 µl; Säule: Aquapore RP 300 4,6x30 mm, 7 µ; Gradientenlösung A: Trifluoressigsäure (TFA), 0,1% (v/v) in Wasser; Gradientenlösung B: TFA, 0,1% (v/v) in Wasser/Acetonitril, 70% (v/v); Gradient: 30 min linear 0–100% B; Durchflußgeschwindigkeit: 0,5 ml/min; Wellenlänge: 215 nm.

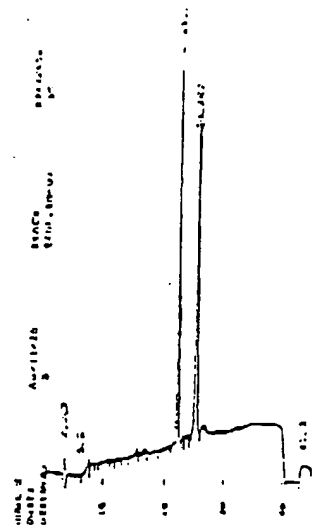


Abb. 2: Spezifität von *Trypsin sequencing grade* in der „reversed phase" HPLC.
Verdauungsansatz: Menge: 100 µg Insulin B_{ox} + 5,6 µg *Trypsin sequencing grade* in 100 µl Tris-HCl-Puffer, 100 mmol/l, pH = 8,5; 18 Stunden bei 37 °C; „reversed phase" HPLC: 10 µl Verdauungsansatz mit Tris-HCl-Puffer auf 100 µl verdünnt; Säule: Polygosil C18; Gradientenlösung A: TFA, 0,1% (v/v) in Wasser; Gradientenlösung B: TFA, 0,1% (v/v) in Wasser/Acetonitril, 30% (v/v); Gradient: 30 min linear 0–100% B; Durchflußgeschwindigkeit: 1 ml/min; Wellenlänge: 215 nm.
Spaltprodukte: 21,7 min Gly(23) – Lys(29),
24,9 min Phe(1) – Arg(22).

Hinweis: Der Inhalt eines Röhrchens kann für mehrere gleichzeitige Ansätze verwendet werden. Bei Wiederholung sollte jedoch auf ein neues Röhrchen zurückgegriffen werden. Dadurch wird größtmögliche Sicherheit und Reproduzierbarkeit gewährleistet und eventuelle Kontamination oder Autolyse vermieden.

Stabilität: Stabil bei +4 °C trocken gelagert. Eine Lösung in Trifluoressigsäure (TFA), 0.01% (v/v) oder HCl, 1 mmol/l, kann maximal 1 Woche bei +4 °C gelagert, verwendet werden.

Anwendung: Lyophilisiertes *Trypsin sequencing grade* in TFA, 0.01% (v/v), oder in HCl, 1 mmol/l, lösen. Zur Vermeidung von Autolyse darf die Inkubationstemperatur 37 °C nicht übersteigen. Die zu sequenzierenden Proteine werden im Verdauungspuffer (Tris-HCl, 100 mmol/l, pH = 8,5) gelöst. Bei schwer löslichen Proteinen sollte dem Puffer SDS, Harnstoff oder Guanidin · HCl in hoher Konzentration zugesetzt werden. Es wird empfohlen bei Verwendung von Harnstoff ebenfalls Methylamin, 20 mmol/l, zuzusetzen. Um eine für das Enzym tolerable Konzentration des Denaturierungsmittels im Verdauungsansatz zu erreichen, muß die Proteinlösung mit Puffer entsprechend verdünnt werden (Tabelle). Die empfohlene Enzymmenge beträgt 1/100 bis 1/20 der Gewichtsmenge an Protein; die Inkubationszeit sollte je nach Enzymmenge zwischen 2 und 18 Stunden bei 37 °C gewählt werden.

Denaturierungsmittel	Konzentration	Enzymaktivität in %
ohne Zusatz (Kontrolle)	—	100
Natriumdodecylsulfat (SDS)	0,001% (w/v)	120
	0.01 % (w/v)	110
	0,1 % (w/v)	105
Harnstoff	0,1 mol/l	86
	0,5 mol/l	86
	1,0 mol/l	90
Guanidin · HCl	0,1 mol/l	85
	0,5 mol/l	95
	1,0 mol/l	97
Acetonitril	1% (v/v)	100
	5% (v/v)	114
	10% (v/v)	134

Tabelle: Inkubation von *Trypsin sequencing grade* 200 µg/ml, mit verschiedenen Denaturierungsmitteln: 6 h bei 25 °C in Tris-HCl, 100 mmol/l, pH = 8,5. Aktivitätsbestimmung des *Trypsin sequencing grade* mit Chromozym® TRY.